Beyond the Biomass
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CHAPTER 22

The microbial loop in soil

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The importance of protozoan feeding on bacteria in nutrient regeneration has been acknowledged in the marine environment since the mid-1960s (Johannes, 1965; Pomeroy, 1970). Later, Williams (1981) demonstrated that, in contrast to earlier beliefs, most of the carbon fixed by minute green algae in the open waters was not channelled along the grazer food chain via zooplankton to fish, but rapidly recycled to CO_2 by bacteria and bacteria-feeding protozoa. This fast turnover of carbon and nutrients through bacteria and protozoa was later termed 'the microbial loop' (Azam et al., 1983).

In soil, the microbial loop has been described as 'the fast flora' around roots (Coleman, 1976; Coleman et al., 1988). Bacteria, utilising root-derived carbon, bind inorganic nutrients transported towards the roots by mass flow and diffusion into bacterial organic matter. The nutrients are thus transported to the root surface, but are inaccessible for root uptake. Grazing by protozoa, mainly naked amoebae, will release about one third of the bacterial nitrogen as ammonium available for uptake by roots. One third is incorporated into the protozoan biomass, and one third is egested by the protozoa in organic forms as parts of cell walls and organelles (Fenchel, 1982a).

This chapter examines the conditions for bacterial consumption prevailing for different groups of protozoa and the importance of the microbial loop as a nutrient regenerator for primary producers in water, in soil under forest and in cultivated soil. It argues that naked amoebae are the most important bacterial grazers in soil and that the importance of the microbial loop in nutrient regeneration will decrease in favour of fungal activities in the order: water > cultivated soil > soil under permanent natural vegetation.

BACTERIAL-PROTOZOAN MIGRATION FROM WATER TO LAND

Unicellular, eukaryotic protozoa were the first bacterial feeders to develop on Earth (Fenchel and Blackburn, 1979). Protozoa accelerated nutrient turnover in the aquatic environment, and this increased nutrient availabity made possible the evolution of larger, multicellular organisms. The bacterial-protozoan complex developed in water and migrated onto land. Both bacteria and protozoa can survive dry conditions in resting states, but they need water to be active. On land, water availability

is often suboptimal for biological processes. To cope with this situation, water transport systems such as the vascular system in plants, fungal hyphae and filamentous bacteria evolved. In unfertilised soil with a permanent vegetation cover, levels of inorganic nutrients are always low, even including periods of high plant uptake. Together with the mycorrhizae, saprophytic fungi close the circulation of nutrients. This leads to a tighter transfer of nutrients within undisturbed terrestrial ecosystems than in cultivated soils and water, where, for example, nitrogen is periodically lost through leaching and denitrification. There are even situations reported where ions were not part of the soil solution, but moved directly from fallen leaves (Herrera et al., 1978) and dying roots (Ritz and Newman, 1985) to living plants, via the mycorrhizae.

Plants on land need more stabilising tissues than plants in water. The supporting tissues are made of lignin, cellulose and other substances, which are more difficult to decompose than the algal cell walls made by polysaccaride polymers. Consequently, saprophytic fungi with enzymes able to release nutrients and carbon from more complex organic substances dominate decomposition in soil, whereas they have a low impact in the aquatic environment.

BACTERIA IN SOIL

Although many common soil bacteria are motile, most bacterial cells in soil are located at, and grow on, surfaces (Clark, 1967; Lynch, 1988). More bacteria are associated with organic surfaces than with surfaces of mineral particles (Marshall, 1976; Lynch, 1988). Most bacterial taxa common in soil have rod-shaped cells as a species characteristic and only a small fraction consists of spherical true cocci (Clark, 1967). However, rod-shaped cells do not make up a large part of the total bacterial community, and their existence is transient. The rods will continue to divide until their food sources are exhausted, whereupon they again become small and rounded (Schaechter, 1968). The rod shape is thus an indication that the cells are actively growing. When viewed by direct microscopy, most bacterial cells in soil are small and rounded. Judging from their shape and size, they are thus mainly in a dormant state. Rod-shaped bacteria in soil are found on fresh litter, on root surfaces and around hyphae.

Drying followed by rewetting triggers bacterial growth activities. On the first day after rain, Clarholm and Rosswall (1980) found that 7% of the total bacterial population in the humus layer of a pine forest consisted of long rods; on the second day this had fallen to 4%. The long rods divided and there was an increase in medium-sized rods which made up 7%, 10% and 11% of the total number of bacterial cells on days 3, 4 and 5, respectively. During the same period, the proportion of long rods reverted to the usually recorded 1-2%. This series of observations indicated a short burst of bacterial production after rain. Bacterial production in soil thus seems to be concentrated in both time and space, leaving large spaces and long periods without production (Nedwell and Gray, 1988).

PROTOZOA AS BACTERIAL CONSUMERS

Based on their mode of locomotion, protozoa are commonly divided into ciliates, flagellates and amoebae. The first two groups are free-swimming and catch bacteria with their cilia or flagella, while amoebae crawl slowly on surfaces, engulfing bacteria by surrounding them with their pseudopodia. With free access to bacteria, many species in all three groups have the same growth rates as bacteria, with divisions every 2-4 hours at room temperature, depending upon the species (Clarholm, 1985b). They are therefore the most potent bacterial feeders in soil (Sandon, 1932). Most soil bacteria are found attached to the soil material. Therefore, naked amoebae feeding on surfaces ought to be more favoured

as bacterial consumers in soil than flagellates and ciliates, which are adapted to feeding in free water. In both forest humus and arable soil, naked amoebae have been shown to cause a decline of elevated bacterial biomass within 1-2 days as a result of grazing (Clarholm, 1981). Ciliates generally need larger bodies of water than the other groups to be active, but some ciliates are better adapted to life in soil. In soil samples investigated in water in the laboratory, small, hypotrich ciliates can be seen 'kicking' off bacteria from a surface with their leg-like ventral cirri, making it possible to catch them with their cilia. Similarly, members of the genus *Colpoda* can grow in small amounts of water with high bacteria concentrations (for example, along a streak of bacteria on an agar plate; pers. obs.).

If changes in numbers of protozoa are to be used to evaluate the potential grazing pressure, then the capacity of individuals to consume bacteria must be known. Flagellates need only a couple of hundred bacteria to divide, while naked amoebae and ciliates need numbers an order of magnitude higher to multiply (Clarholm, 1985b). Each naked amoeba or ciliate thus represents a grazing pressure at least 10 times greater than that represented by a flagellate. The relative importance of the three groups as bacterial consumers in the aquatic and terrestrial environments can be predicted from their modes of feeding. In open waters, where the bacterial concentration is too low to support multiplication of ciliates, flagellates are the dominant consumers (Fenchel, 1982b). Ciliates dominate in more productive aquatic situations near the sediment surface, where they tend to filter-feed on bacteria, but some also ingest flagellates and small ciliates. Amoebae are scarce because of the absence of solid surfaces on which to crawl. Similarly, in soil, flagellates dominate protozoan activities where bacterial production is low (for example, in soil without plants, and at the start of a flush of bacterial activity after rain). Where there is a sizable bacterial production, naked amoebae are the dominant consumers.

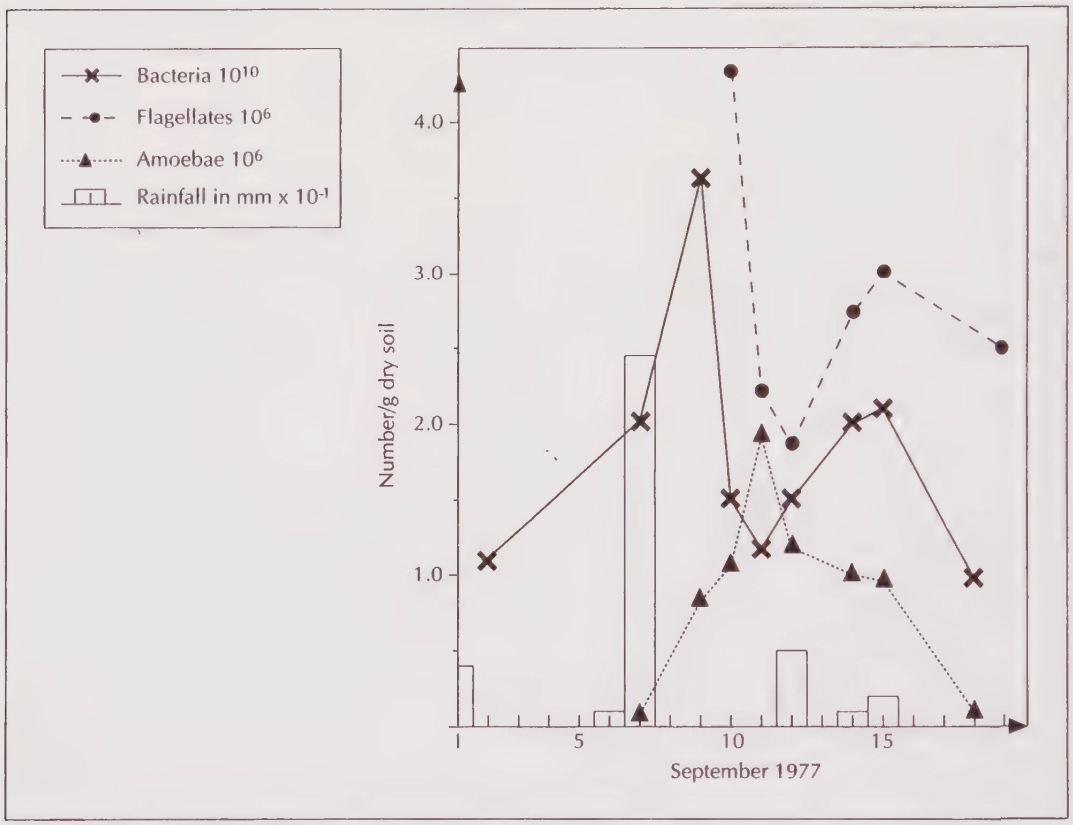
The relationship between naked amoebae and flagellates can be demonstrated with data from the humus layer of a pine forest (*see* Figure 22.1 *overleaf*), where bacteria and flagellates started to increase immediately after rain and peaked after 2-3 days; flagellates then decreased 1 day after the bacteria had started to decrease. The numbers of naked amoebae increased more slowly and peaked at day 4-5 after rain (*see* Figure 22.1). The same relationship between bacteria, flagellates and naked amoebae was reported in a 1-year study in which daily estimates of protozoa and bacteria were made at Rothamsted, UK in a long-term, manured, arable field kept under fallow (Cutler et al., 1922). An inverse relationship between numbers of bacteria and naked amoebae was found in 86% of the daily observations (Cutler et al., 1922). In the same study, flagellates were observed to be dependent upon a bacterial production to sustain themselves, but they did not reduce the numbers of bacteria (Darbyshire et al., 1993).

MICROCOSMS AND THE REAL WORLD

One way used to study the effects of microbial interactions has been to build gradually more complex microcosms by adding organism groups to sterilised soil. Increased amounts of inorganic nitrogen have been reported in soil in microcosms with both bacteria and protozoa, compared with soil containing only bacteria (Coleman et al., 1977; Kuikman and van Veen, 1989). These results are presumably correct for the situation investigated, but how do the created situations relate to the real world? Obvious differences are the absence of fungi and larger animals and the presence of a new pool of organic matter — the necromass of the organisms killed by sterilisation.

Increased nitrogen mineralisation after the addition of flagellates only to sterilised soil reinoculated with bacteria has been reported (Gerhardson and Clarholm, 1986; F. Verhagen, pers. comm.). When enumerating bacteria in reinoculated soil using direct microsocopy (Clarholm, 1985a), it was clear that the reintroduced bacteria had not managed to colonise the soil particles in the same way as the native bacteria had in unsterilised soil. The bacteria were larger and fewer than normally observed in the same

Figure 22.1 Daily estimates of numbers of bacteria, naked amoebae and flagellates in the humus layer of a pine forest after rain



soil when not sterilised. Furthermore, they tended to float about, rather than being attached to surfaces (Clarholm, 1985a). Reinoculation with bacteria thus created an aquatic-like situation in the soil. In this situation, flagellates should be more favoured as bacterial grazers than in unsterilised soil, where most bacteria are attached to surfaces.

Microcosm experiments performed to date have shown that:

- bacteria exceeding the carrying capacity of the soil (van Veen et al., 1985) will be quickly consumed by the grazers most suited to the situation
- nitrogen and other nutrients are released during this process
- a carbon source for bacteria, such as roots, must be present for protozoa to be continously active (Kuikman et al., 1990)

However, an assessment of the importance of these interactions for nutrient regeneration in the field cannot yet be made because too few relevant studies have been conducted. In a barley field, short-term decreases in numbers of bacteria corresponded to 10% and 17% of the plant uptake of nitrogen over

the same 2-day periods, if one assumed that one third of the bacterial nitrogen became available for plant uptake (Clarholm, 1989), the other sources being fertiliser and nitrogen released by decomposer fungi. A model of nitrogen circulation in the same treatment indicated that 14% of the harvested nitrogen was made available through faunal activities, mainly by protozoa (Paustian et al., 1990).

THE IMPORTANCE OF THE MICROBIAL LOOP IN WATER AND ON LAND

In the aquatic environment, where fungi are of little importance, bacteria and protozoa play the dominating role in decomposition and nutrient mineralisation (Azam et al., 1983; Wangersky, 1984). The traditional view of the situation in the sea, where nutrients were thought to be regenerated mainly in the sediment, has gradually changed with the recognition of the activities on organic particles and in secondarily formed aggregates floating in the free water. These particles are biological hot spots and it is estimated that only 5% of the organic matter produced will eventually reach the bottom of the sea (Wangersky, 1984). Thus, most of the nutrients are regenerated within the water column via the microbial loop (Azam et al., 1983; Münster and Chróst, 1990)

A natural soil undisturbed by humans contains mycorrhizal roots and heterotrophic decomposer fungi in addition to bacteria and protozoa. The fungi form two types of pipeline systems, which differ in their sources of energy. Mycorrhizae are supplied from above-ground plant parts, while decomposer fungi obtain their energy mainly through the decomposition of dead plant parts of different ages. The energy sources for bacteria in soil are less clear. Their physiology indicates a dependence upon low-molecular-weight substrates. The most obvious sources are fresh above-ground litter and root-derived carbon. A third source can be envisaged around decomposer fungi when enzymes are secreted from the hyphae to act on the organic matter. The resulting smaller molecules may be utilised by both the decomposer fungi and bacteria.

SPATIAL CONSTRAINTS

Fungal hyphae can grow past air pockets and nutrients are transported within hyphae to support fungal growth on suitable carbon sources. The possibility of nitrogen translocation to suitable carbon sources is an important factor in coniferous forests, where the C/N ratios of the fresh organic matter are much wider than in water and arable soil and where there are no burrowing animals mixing the substrates. The importance of upward translocation of nutrients by fungi was demonstrated in two coniferous forest ecosystems where the transport of nitrogen from the mineral soil to the pine needle litter on the forest floor was estimated to be 9 kg N/ha (Hart and Firestone, 1991) and 4 kg kg N/ha (Fahey et al., 1985), representing about half the plant uptake.

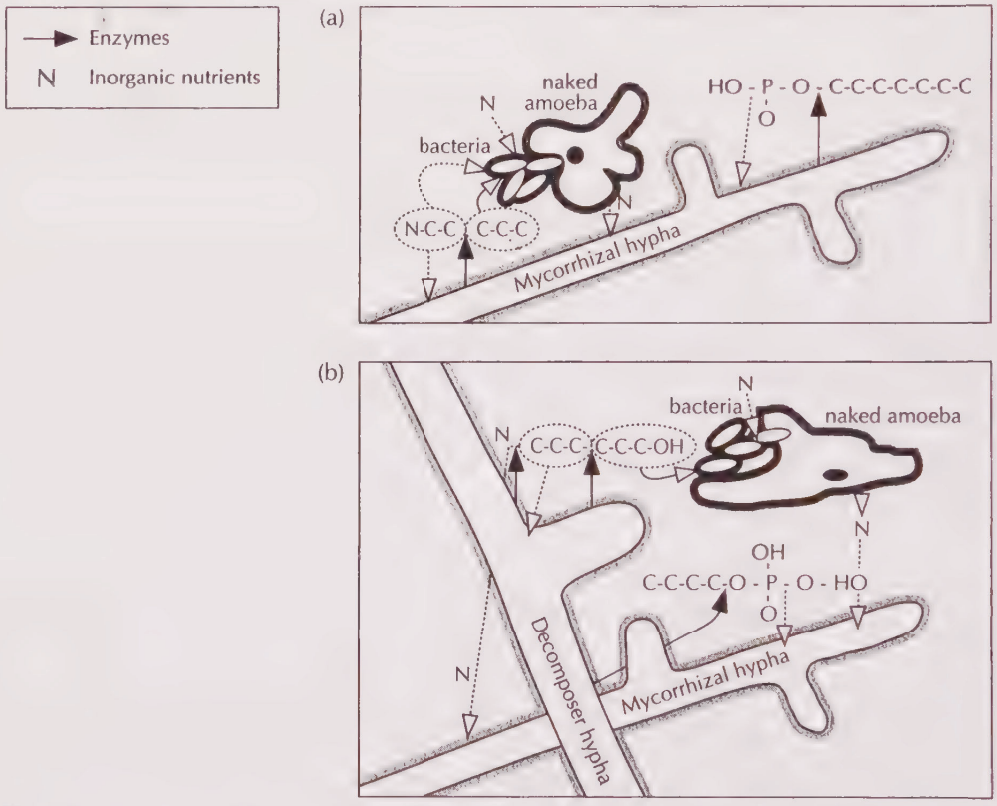
Bacteria are attached to surfaces and caught in isolated bodies of water, the sizes of which are determined by the structure of the soil (Foster, 1988). The heterogeneous distribution of organic matter and available nutrients in soil will cause problems for bacteria in terms of the supply of suitable energy, resulting in long periods of inactivity.

THE RELATIONSHIP BETWEEN THE MICROBIAL LOOP AND FUNGI

Few parts of the fine-root system of coniferous plants are non-mycorrhizal. However, little information on mycorrhiza-derived carbon supporting bacterial growth is available (Ingham and Molina, 1991).

Significantly increased numbers of naked amoebae and ciliates within mats of the ectomycorrhizal basidiomycete, *Hysterangium setchellii* (Cromack et al., 1988), indicated the presence of an available carbon source for bacteria. Mycorrhizal fungi may be better decomposers of organic material than has been assumed (Trojanowski et al., 1984; Dighton et al., 1987) (*see* Figure 22.2a). There is also the

Figure 22.2 Possible interactions of decomposer fungi, mycorrhizal fungi and the 'microbial loop' in nutrient mineralisation from organic matter and uptake by the mycorrhizae



(a) The mycorrhizal fungus will obtain its carbon from the plant. Its hypha is able to produce catalytic enzymes, such as phosphatases, which will be used to release nutrients to be taken up by the mycorrhiza and forwarded to the plant. Carbon in smaller units than existed before the fungal attack will be used by bacteria for growth and nutrient immobilisation. Protozoa will release part of the immobilised nutrients through grazing.

b) If the mycorrhizal fungus functions only as a thin root without catalytic enzymes, then it has to rely on the activities of a decomposer fungus to obtain inorganic nutrients. The decomposer fungus produces extracellular enzymes to obtain a carbon source and nutrients for its own growth and metabolic activities. In that process, inorganic nutrients will also be liberated for mycorrhizal uptake. Bacteria will scavenge for carbon substrates around the decomposer fungus and compete with the mycorrhiza for nutrients. Protozoan grazing of bacteria will release nutrients, otherwise locked up in bacteria, for uptake by the mycorrhiza.

possiblity of a four-partner situation whereby saprophytic decomposer fungi are intermingled within the mycorrhizal mat (*see* Figure 22.2b). The decomposer fungus would release enzymes to cut long carbon chains, providing substrate both for itself and for scavenging bacteria, close to the enzyme-producing hypha. At the same time, nutrients released by enzymes from the decomposer hyphae would be taken up by the mycorrhizae but also by the bacteria present. Protozoan grazing on bacteria would again release part of the nitrogen for mycorrhizal uptake. But what might have caused the mycorrhizal mat to develop where it did? Substrate quality of the organic matter is probaby involved, but which organisms released the energy for the bacteria and the nutrients for the mycorrhiza? A root with a mycorrhiza with decomposer abilities might be the most efficient solution for plants in the terrestrial habitat. The bacteria-protozoa complex in soil would then be a relict with regard to the decomposition of organic matter.

In cultivated soils, humans have changed the naturally stable terrestrial situation through frequent mixing of the soil, large inputs and exports of nutrients, and monocultural crop management practices which include long periods during which fertile soils are left with no plant cover and thus no root uptake. In relation to the importance of bacterial-protozoan interactions, this is a situation which lies between the aquatic and the natural, terrestrial situation, since mixing the soil increases the possibilities for bacteria to reach suitable substrates for growth. Decreased mixing of soil in experiments involving minimum tillage has also been reported to increase the proportion of fungi in relation to bacteria (Beare et al., 1992).

Mycorrhizae play an insignificant role in conventionally fertilised agricultural soils. Decomposer fungi are generally more important, but their relative contribution to the microbial biomass seems to vary. In arable soils derived from forests, which are common in, for example, Sweden and Germany, the dominance of fungi is strong in the microbial biomass (Anderson and Domsch, 1973; Schnürer et al., 1985), while bacteria have been reported to dominate the microbial biomass in arable soils in the Netherlands (Bloem et al., 1992). The arable soils in the Netherlands are derived mainly from the sea or lakes, and at least part of the organic matter has an aquatic history. Another difference is the common use of fumigation in the Netherlands to fight pathogens. Nitrogen additions to boreal forest soils are reported to change the size and activity of the microbial biomass; long-lasting reductions in the fungal-dominated microbial biomass, as well as a reduced CO_2 evolution, have been reported in boreal forest soils after nitrogen fertilisation (Söderström et al., 1983; Nohrstedt et al., 1989).

THE IMPORTANCE OF THE MICROBIAL LOOP IN MINERALISATION

In nature, bacteria and protozoa generally occur together and they should be viewed as a unit in relation to nutrient turnover. The degree of importance of the microbial loop in terms of the amount of nutrients mineralised depends upon the amount of bacteria produced. In this respect, the amount of bacterial biomass exceeding carrying capacity, which is immediately turned over, constitutes the interesting part of the bacteria, since the remainder is protected from grazing. It is almost impossible to measure the bacterial production above carrying capacity because it is unevenly distributed and rapidly turned over. If the amount of carbon available for bacteria could be estimated, indirect calculations could be made. It is, however, only the input of above-ground litter that is known in most ecosystems. Too little is known at present about the amounts of root-derived carbon and how the activities of saprophytic fungi contribute to the pool of carbon available for bacteria to determine their absolute contribution to nurient regeneration.

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References

Anderson, J.P.E. and Domsch, K.H. 1973. Quantification of bacterial and fungal contributions to soil respiration. *Archiv für Mikrobiologie* 93: 113-27.

Azam, F., Fenchel ,T., Field, J., Gray, J., Meyer-Reil, L. and Thingstad, F. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* 10: 257-63.

Beare, M.H., Parmelee, R.W., Hendrix, P.W., Cheng, W., Coleman, D.C. and Crossley, D.A. 1992. Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. *Ecological Monographs* 62: 569-91.

Bloem, J., De Ruiter, P.C., Koopman, G.J., Lebbink, G. and Brussard, L. 1992. Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management. *Soil Biology and Biochemistry* 24: 655-65.

Clarholm, M. 1981. Protozoan grazing of bacteria in soils: Impact and importance. *Microbial Ecology* 7: 343-50.

Clarholm, M. 1985a. Interactions of bacteria, protozoa and plants leading to mineralisation of soil nitrogen. *Soil Biology and Biochemistry* 17: 181-87.

Clarholm, M. 1985b. Possible role for roots, bacteria, protozoa and fungi in supplying nitrogen to plants. In Fitter, A.H. and Usher, M. (eds) *Ecological Interactions in Soil*. Oxford, UK: Blackwell .

Clarholm, M. 1989. Effects of plant-bacterial-amoeban interactions on plant uptake of nitrogen under field conditions. *Biology and Fertility of Soils* 8: 373-78.

Clarholm, M. and Rosswall T. 1980. Biomass and turnover of bacteria in a forest soil and a peat. *Soil Biology and Biochemistry* 12: 49-57.

Clark, F.E. 1967. Bacteria in soil. In Burges, A. and Raw, F. (eds) *Soil Biology*. London, UK: Academic Press.

Coleman, D.C. 1976. A review of root processes and their influence on soil biota in terrestrial ecosystems. In Anderson, J.M. and Macfayden, A. (eds) *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes*. Oxford, UK: Blackwell.

Coleman, D.C., Cole, C.V., Anderson, R.V., Blaha, M., Campion, M.K., Clarholm, M., Elliott, E.T., Hunt, H.W., Shaefer, B. and Sinclair, J. 1977. An analysis of rhizosphere-saprophytic interactions in terrestrial ecosystems. *Ecological Bulletins (Stockholm)* 25: 299-309.

Coleman, D.C., Crossley, D.A.J., Beare, M.H. and Hendrix, P.F. 1988. Interactions of organisms at root/soil and litter/soil interfaces in terrestrial systems. In Edwards, C.A., Stinner, B.R., Stinner, D. and Rabatin, S. (eds) *Biological Interactions in Soil*. Amsterdam, Netherlands: Elsevier.

Cromack, K.J., Fichter, B.L., Moldenke, A.M., Entry, J.A. and Ingham, E.R. 1988. Interactions between soil animals and ectomycorrhizal fungal mats. *Agriculture, Ecosystems and Environment* 24: 161-68.

Cutler, D.W., Crump, L.M. and Sandon, H. 1922. A quantitative investigation of the bacterial and protozoan population of the soil, with an account of the protozoan fauna. *Philosophical Transactions of the Royal Society of London (Series B)* 211: 317-50.

Darbyshire, J.F., Zwart, K.B.and Elson, D.A. 1993. Growth and nitrogeneous excretion of a common soil flagellate, *Cercomonas* sp. *Soil Biology and Biochemistry* (in press).

Dighton, J., Thomas, E.D. and Latter, P.M. 1987. Interactions between tree roots, mycorrhizas, a saprophytic fungus and the decomposition of organic substrates in a microcosm. *Biology and Fertility of Soils* 4: 145-50.

Fahey, T.J., Yavitt, J.B., Pearson, J.A. and Knight, D.H. 1985. The nitrogen cycle in lodgepole pine forests, southeastern Wyoming. *Biogeochemistry* 1: 257-75.

Fenchel, T. 1982a. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. *Marine Ecology Progress Series* 8: 225-31.

Fenchel, T. 1982b. Ecology of hetertrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. *Marine Ecology Progress Series* 9: 35-42.

Fenchel, T. and Blackburn, T.H. 1979. *Bacteria and Mineral Cycling*. London, UK: Academic Press.

Foster, R.C. 1988. Microenvironments of soil microorganisms. *Biology and Fertility of Soils* 6: 189-203.

Gerhardson, B. and Clarholm, M. 1986. Microbial communities on plant roots. In Jensen, V., Kjøller, A. and Sørensen, L.H. (eds) *Microbial Communities in Soil*. Amsterdam, Netherlands: Elsevier.

Hart, S. and Firestone, M.K. 1991. Forest floor-mineral soil interactions in the internal nitrogen cycle of an old-growth forest. *Biogeochemistry* 12: 103-27.

Herrera, R., Merida, T., Stark N. and Jordan C. 1978. Direct phosphorus transfer from leaf litter to roots. *Naturwissenschafter* 65: 208-09.

Ingham, E.R. and Molina, R. 1991. Interactions among mycorrhizal fungi, rhizosphere organisms, and plants. In Barbosa, P., Krischik, V. and Jones, C.G. (eds) *Microbial Mediation of Plant-Herbivore Interactions*. Chichester, UK: John Wiley

Johannes, R.E. 1965. Influence of marine protozoa on nutrient regeneration. *Limnology Oceanography* 10: 434-42.

Kuikman, P.J., van Elsas, J.D., Jansen , A.G., Burgers, S.L.G.E. and van Veen, J.A. 1990. Population dynamics and activity of bacteria and protozoa in relation to their spatial distribution in soil. *Soil Biology and Biochemistry* 22: 1063-73.

Kuikman, P.J. and van Veen, J.A. 1989. The impact of protozoa on the availability of bacterial nitrogen to plants. *Biology and Fertility of Soils* 8: 13-18.

Lynch, J.M. 1988. The terrestrial environment. In Lynch, J. and Hobbie, J. (eds) *Microorganisms in Action: Concepts and Applications in Microbial Ecology*. Oxford, UK: Blackwell .

Marshall, K.C. 1976. *Interfaces in Microbial Ecology*. Cambridge, Massachusetts, USA: Harvard University Press.

Münster, O. and Chróst, R.J 1990. Origin, composition, and microbial utilisation of dissolved organic matter. In Overbeck, J. and Chróst, R.J. (eds) *Aquatic Microbial Ecology: Biochemical and Molecular Approaches*. New York, USA: Brock/Springer.

Nedwell, F.B. and Gray, T.R.G. 1988. Soils and sediments as matrices for microbial growth. In Fletcher, M., Gray, T.R.G. and Jones, J.G. (eds) *Ecology of Microbial Communities*. Cambridge, UK: Cambridge University Press.

Nohrstedt, H.-O., Arnebrant, K., Bååth, E. and Söderström, B. 1989. Changes in carbon content, respiration rate, ATP content and microbial biomass in nitrogen-fertilised pine forest soils in Sweden. *Canadian J. Forestry Research* 19: 323-28.

Paustian, K., Andrén, O., Clarholm, M., Hansson, A.-C., Lagerlöf, J., Lindberg, T., Pettersson, R. and Sohlenius, B. 1990. Carbon and nitrogen budgets of four agro-ecosystems with annual and perennial crops, with and without N fertilisation. *J. Applied Ecology* 27: 60-84.

Pomeroy, L.R. 1970. The strategy of mineral cycling. *Annual Review of Ecological Systems* 1: 171-90.

Ritz, K. and Newman, E. 1985. Evidence for rapid cycling of phosphorus from dying roots to live plants. *Oikos* 45: 174-80.

Sandon, H. 1932. *The Food of Protozoa*. Cairo, Egypt: MIRS-Sokkar Press.

Schaechter, M. 1968. Growth: Cells and populations. In Mandelstam, J. and McQuillen, K. (eds) *Biochemistry of Bacterial Growth*. Oxford, UK: Blackwell.

Schnürer, J,. Clarholm, M. and Rosswall, T. 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. *Soil Biology and Biochemistry* 17: 611-18.

Söderström, B., Bååth, E. and Lundgren, B. 1983. Decrease in soil microbial activity and biomasses owing to nitrogen amendments. *Canadian J. Microbiology* 29: 1500-06.

Trojanowski, J,. Haider, K. and Hutterman, A. 1984. Decompostion of ^{14}C-labelled lignin, holocellulose and lignocellulose by mycorrhizal fungi. *Archiv für Mikrobiologie* 139: 202-06.

van Veen, J., Ladd J. and Amato, M. 1985. Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [$^{14}C(U)$]glucose and [^{15N}]$(NH_4)_2SO_4$ under different moisture regimes. *Soil Biology and Biochemistry* 17: 747-56.

Wangersky, P.J .1984. Organic particles and bacteria in the ocean. In Hobbie, J.E and Williams, P.J. Le B. (eds) *Hetertrophic Activity in the Sea.* New York, USA: Plenum Press

Williams, P.J . Le B. 1981. Incorporation of microheterotrophic processes into the classic paradigm of the planctonic food web. *Kieler Meerforschung, Sonderheft* 5: 1-28.